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Configuration analysis of unsaturated hydroxy fatty acids

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ABSTRACT

Stereoisomers of methyl ricinoleate, methyl isoricinoleate, and related methyl esters of bis-homoallylic hydroxy fatty acids were reacted with (*R*)- and (*S*)-α-naphthylethyl isocyanate to form diastereomeric carbamates that separated well on silica gel high-performance liquid chromatography. Proton nuclear magnetic resonance shifts of the carbomethoxymethyl protons were used to assign configuration to the alcohol component of the carbamate derivative. Combining high-performance liquid chromatography with proton nuclear magnetic resonance spectroscopy may allow one to determine the configuration of naturally occurring hydroxy fatty acids as well as to determine configurational purity.

INTRODUCTION

Separations of selected chiral hydroxy fatty acids and esters by gas–liquid chromatography (GC) and high-performance liquid chromatography (HPLC) has been summarized recently [1]. In particular, α-hydroxy acids, and less frequently β-hydroxy acids, have been resolved using chiral stationary phases or by derivatization with a common chiral derivatizing agent (CDA) to afford separable diastereomers. In addition, chiral allylic [2,3] and propargylic alcohols [4] can be distinguished by conversion to esters of (*R*)- or (*S*)-α-methoxytrifluoromethylphenylacetic acid and observing the shifts of the methoxyl protons.

Ricinoleic acid, (*R*)-12-hydroxy-(*Z*)-9-octadecenoic acid, and isoricinoleic acid, (*S*)-9-hydroxy-(*Z*)-12-octadecenoic acid, are industrially important natural products [5] whose syntheses and stereochemistry have been reviewed [6]. We are unaware of a simple method by which to distinguish or separate enantiomers of such homoallylic and bis-homoallylic structures. We report here that diastereomeric carbamates of the methyl esters of these alcohols formed from (*R*)- or (*S*)-α-naphthylethyl-isocyanate (NEI) as the CDA are cleanly separated by HPLC with a silica gel column. Moreover, the ^{1}H NMR shifts of CH_3O_2C–can be employed to characterize the alcohol's configuration.

EXPERIMENTAL☆

GC was performed with a Supelcowax column (30 m × 0.25 mm I.D.) using a Chrompack-Packard Model 438A chromatograph operating with helium carrier gas at 18 cm/s and a 50:1 split ratio (the subject diastereomers were not resolved with this column). HPLC was performed using a Spectra-Physics SP8800 pump, SP8480 XR scanning UV detector and SP4290 integrating recorder with a silica gel column (15 cm × 0.25 in.) from Supelco. Infrared spectra were recorded with a Perkin-Elmer 1310 spectrophotometer using 3% solutions in carbon tetrachloride. Mass spectra were obtained with a Hewlett-Packard HP-5995 GC–mass spectrometry (MS) system using the direct probe. ^{1}H NMR spectra were obtained with a JEOL JNM-GX 400 Fourier transform (FT) NMR spectrometer with [2]

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chloroform as the solvent and tetramethylsilane as internal standard. The solvents employed were HPLC-grade, and the tetrahydrofuran was dried initially over KOH and then distilled from lithium aluminum hydride with storage in a stoppered bottle containing molecular sieve 4A. Thin-layer chromatography (TLC) was performed with standard analytical plates of silica gel from Analtech. Silica gel (60–200 mesh) for open column chromatography was purchased from Baker.

D-Methyl ricinoleate that was >98% pure by GC, was a generous gift of Dr. Robert Benedict of this laboratory, and D-methyl isoricinoleate was islated from the seeds of *Holarrhena antidysenterica* (NU-46607), a gift of Dr. Robert Kleiman of the Northern Regional Research Center, Peoria, IL, USA, using essentially the procedure described previously [7] to obtain GC-pure material. The other bis-homoallylic hydroxy fatty acids described herein were prepared in conjunction with other research, and their syntheses will be described elsewhere. Each hydroxylated fatty acid, natural and synthetic, was employed as its methyl ester (>98% GC-pure) and had spectral characteristics (IR, ^{1}H NMR, ^{13}C NMR, and MS) consistant with its structure. (*R*)- and (*S*)-α-NEI that was 98% pure by label was purchased from Aldrich.

Synthesis of diastereomeric carbamate derivatives

The general procedure was to place 100 μl each of the (*R*)- or (*S*)-α-NEI and the unsaturated hydroxy fatty acid methyl ester in 1 ml of toluene. This was heated at 90–95°C under nitrogen for 1 h. The mixture was concentrated on a flash evaporator and then placed on top of a column of 60–200-mesh silica gel (1 g) in a disposable pipet. Portions of 10 ml of hexane–chloroform mixtures were used to chromatograph the crude product, and the elution was monitored by TLC. Unreacted isocyanate eluted first; the carbamates eluted with 20–30% chloroform. For example, in this manner was obtained the adduct of racemic methyl 9-hydroxy-(*Z*)-(12)-octadecenoate a colorless oil that solidified on standing: IR 3460, 3010, 1740, 1715 cm^{-1}, ^{1}H NMR δ 7.4-8.2 (m, 7H, naphthyl H), 5.65 (m, 1H, NHC*H*CH$_3$), 5.4 (m, 2H, RCH=CHR′), 5.1 (m, 1H, HCO), 3.65 (*R*,*R*) and 3.66 (*R*,*S*) (2s, 3H, CH$_3$O$_2$C–), 2.30 (m, 2H, CH$_2$C=O), 2.03 (m, 2H, CH$_2$C=C), 1.63 (d, J = 6.2 Hz, *ca.* 3H, C*H*$_3$CH), 1.5 (m, *ca.* 2H, C*H*$_2$CH$_2$C=O), 1.2 [CH$_5$ envelope (env.)], 0.85 [broad triplet (bt), 3H, C*H*$_3$CH$_2$] ppm; MS *m*/*e* (relative abundance) 509 [M]$^+$ (0.07), 294 (0.65), 215 (0.71), 214 (0.30), 200 (0.68), 182 (0.29), 155 (1.00). HPLC data are presented in Table I. The other carbamate derivatives gave analogous data.

RESULTS AND DISCUSSION

Racemic methyl isoricinoleate (**1**, Fig. 1), was synthesized by us in connection with another project [8], and was converted to a mixture of diastereomeric carbamates by reaction with (*S*)-α-NEI.

TABLE I

CHROMATOGRAPHIC DATA FOR α-NAPHTHYLETHYLCARBAMATES[a]

Alcohol	k' values	α	^{1}H NMR shifts (ppm) for –CO$_2$CH$_3$		
			HPLC-1[b]	HPLC-2[b]	$\Delta\delta$(2−1)
1	5.07, 5.95	1.17	3.654(*R**,*R**)	3.665(*R**,*S**)[c]	0.011
2	6.54, 7.80	1.19	3.639(*R**,*R**)	3.650(*R**,*S**)[d]	0.011
3	3.73, 4.85	1.30	3.665(*R**,*R**)	3.660(*R**,*S**)[c]	−0.005
4	5.10, 5.76	1.13	3.656(*R**,*S**)	3.623(*R**,*R**)[d]	−0.034
5	4.73, 5.08	1.07	3.637(*R**,*S**)	3.625(*R**,*R**)[d]	−0.012

[a] Silica gel analytical column using hexane–ethyl acetate–tetrahydrofuran (95:5:1). Stereochemical designators are for relative configuration. Note that the designator for the alcohol component of 1 and 2 is opposite from that of 3–5. k' = Capacity factor; α = separaton factor.

[b] HPLC-1 and -2 refer to elution order.

[c] Stereochemistry known (natural product).

[d] Stereochemistry inferred from relative ^{1}H NMR shifts of –CO$_2$CH$_3$ signals.

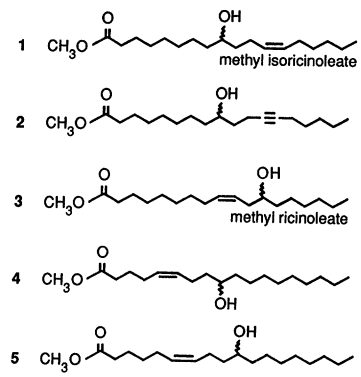

Fig. 1. Structures of alcohols the α-NEI derivatives of which are discussed. The racemic structures are indicated.

These were cleanly separated by a silica gel HPLC column (Fig. 2). The elution order of the diastereomers (Table I) was determined using a sample of isoricinoleic acid of known configuration that we had obtained by isolation from seeds of *Holarrhena antidysenterica* [7]. A synthetic precursor to the racemic acid, the corresponding acetylenic structure **2**, similarly provided chromatographically separated diastereomers. The elution order for **2** was judged to be the same as for **1** since the ^{1}H NMR singlets for $-CO_2CH_3$ of the diastereomers bore the same relationship for **2** as for **1**, namely the methyl singlet was shifted upfield by 0.01 ppm in the earlier eluting diastereomer in both cases.

D-Methyl ricinoleate (**3**) a commercially available natural product with (*R*)-configuration was converted to the analogous derivatives using each enantiomer of α-NEI. Again chromatographic separation was excellent (Fig. 3) though the shift difference for the $-CO_2CH_3$ was much smaller. For both ricinoleate and isoricinoleate structures the *R*R** diastereomer (the asterisk denotes relative configuration of the two asymmetric centers of the derivative) elutes first. We also derivatized samples of methyl 9-hydroxy-(*Z*)-5-octadecenoate (**4**) and methyl 10-hydroxy-(*Z*)-6-octadecenoate (**5**). These compounds (racemic) were available from related work and are bishomoallylic alcohols like methyl isoricinoleate. The relevant diastereomers were again resolved by silica gel HPLC (Table I).

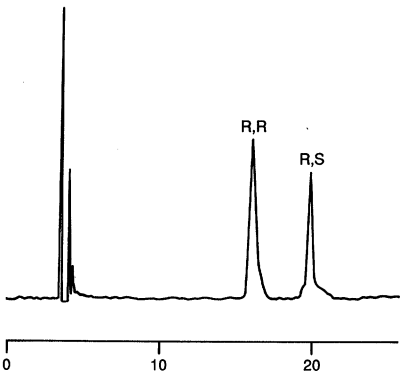

Fig. 2. Chromatogram of carbamates obtained from *rac.*-methyl isoricinoleate (**1**) and (*S*)-α-NEI using silica gel, 1.0 ml/min, hexane–ethyl acetate–tetrahydrofuran (95:5:1).

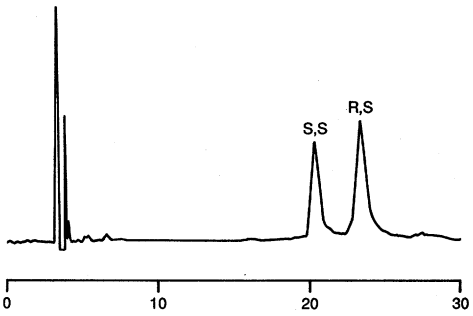

Fig. 3. Chromatogram of carbamates obtained from D-methyl ricinoleate, (*R*)-**3** and (*R*)- and (*S*)-α-NEI using silica gel, 1.0 ml/min, hexane–ethyl acetate–tetrahydrofuran (95:5:1).

The principal solution conformation of diastereomeric carbamates of type **6** (Fig. 4) is such as to place the substituents R_1 and R_2 of the chiral alcohol on either side of a central plane defined by the carbamate structure and its attached asymmetric centers [9]. In **6A** R_1 will on average experience more of the diamagnetic anisotropy associated with the large 1-naphthyl group and conversely for **6B** in which R_2 is placed in that situation. Although the $-OCH_3$ is remote from the aryl ring (9 bonds from the asymmetric carbon of methyl isoricinoleate, for example), shift differences of 0.01–0.04 ppm were observed for these singlets. Similar shift differences of remotely situated protons in diastereomeric

Fig. 4. Principal solution confirmation of simple diastereomeric carbamates [9]; Np = 1-Naphthyl.

amides have been noted and used to make configurational assignments [10]. For compounds **1** and **3**, assignments of configuration of the alcohol components by ^{1}H NMR was not necessary since the (configurationally pure) natural products were available and could be derivatized directly for HPLC comparison. However, we noted that such an assignment was indeed consistent with that predicted from the ^{1}H NMR spectra of the derivatives.

The shift differences for **3**, **4** and **5** were determined in C^2HCL_3 solutions of mixtures of the diastereomers biased by appropriate HPLC collections to allow the signal assignment to be related to elution pattern. Assignment of stereochemistry based on ^{1}H NMR of a single diastereomer would be doubtful because of the small shift differences. Nevertheless, since the major solution conformation of such compounds would not be expected to deviate from that of secondary alcohols not bearing a terminal carbomethoxy group (Fig. 4), we were able to make assignment of stereostructure using NMR having both diastereomers on hand. For an unknown hydroxy fatty acid, therefore, one would prepare the carbamates from each of the enantiomeric CDAs and determine configuration by ^{1}H NMR. The configurational purity, however, could be determined better by HPLC.

No simple relationship seems to exist for the observed elution orders and the structures of the major solution conformers. The elution orders are reversed from that of, for example, methyl isoricinoleate adducts when the unsaturation is inserted between the carbamate and carbomethoxy groups as in **4** and **5**. This probably is a reflection of the multiplicity of adsorption sites available in these compounds; both the ester and carbamate groups can become associated with the stationary phase. Nevertheless, judging from these observations, one may expect to separate and characterize the α-naphthylethylcarbamates of both homo- and bis-homoallylic hydroxy fatty acids.

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